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DEPARTMENT OF THE ARMY
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FAST METHOD OF DIAGNOSIS OF PERTUSSIS DURING
THE CATARRHAL PERIOD OF THE DISEASE

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The method consists in using the technique of fluorescent antibodies which makes it possible in less than one hour to diagnose pertussis during the catarrhal period of the disease. This method is more sensitive during this period than the technique of culturing the nasopharyngeal mucus on Bordet-Gengou's medium.

*

Several methods of bacteriological diagnosis have been described since the discovery of the coccus bacillus of pertussis by Bordet and Gengou in 1906, aiming at recognizing this disease during its catarrhal phase. The difficulties of diagnosing this disease during this phase are numerous and well known.

In 1916 two Danish authors, H. Meyer and Mrs. P. Chie-vitz, basing themselves on the principle described by Mauritzen, directly seeded a Bordet-Gengou medium in a Petri dish with Pfluegge's droplets expelled during a fit of coughing.

In 1928, J. Marie replaced the human blood of the original Bordet-Gengou medium with defibrinated horse blood, and obtained the same results.

In 1937, MacLean proposed adding 4 to 5 drops of a penicillin solution containing 1000 units/cm³ in order to prevent the development of other germs.

The methods of seeding are delicate, and are of major interest because it is on them that the success of the culture technique depends. In the case of Mauritzen's procedure, Pfluegge's droplets were directly inoculated into Bordet-Gengou's medium; in 1940 Bradford and Salvin developed a method of direct sampling of nasopharyngeal mucus by a swab, and in 1955 Herzog proposed the aspiration of nasopharyngeal mucus by means of a probe. Despite the very considerable improvement brought about by this method of sampling, it was always necessary to wait 48-72 hours in order to identify the colonies of Bordetella Pertussis in the culture.

The hemogram method does not give valid results in pertussis, particularly during the catarrhal period. In effect, it was found in the department of Prof. Julien Marie that 80% of the patients suffering from pertussis had a blood composition indicating the presence of the disease, and that 70% of those not afflicted with pertussis had a composition similar to the former even though they had only a minor cough. Moreover, the hematological modifications are absent during the first week following the beginning of the cough; and at least 50% of the patients with pertussis show no significant modification of the blood composition.

A new method was applied in 1956 by de Repentigny and Frappier in Canada, based on the technique of fluorescent antibodies which makes it possible to demonstrate the surface antigens of the germ. (This method is becoming more and more widespread for the rapid identification of the infectious diseases; the result is certain and is obtained almost immediately.)

In 1960 this method was applied to pertussis by Donaldson, Whitaker and Nelson, and in 1961 by Rendring, Eldering et al, and the diagnosis of pertussis was established by identifying the presence of pertussis bacilli in the smears of the nasal mucus of children suffering from this disease.

The advantage of this method is that the results are known in less than one hour after the taking of the sample.

This technique consists of the following steps:

- 1) A smear of nasopharyngeal mucus of the child suspected of pertussis is spread on slides; this mucus is taken from the posterior wall of the pharynx by the aspiration method, using a rubber probe.

2) The smear is allowed to dry in air, and is then fixed with acetone for ten minutes.

3) The smear is covered with 2-3 drops of antibody solution. The mixture is then left for 20 minutes in a humid atmosphere. (The antibody solution consists of a solution of globulins of a rabbit hyperimmunized against pertussis, having a high agglutinin titer (1/20,000), conjugated with fluorescein isothiocyanate which gives it a greenish yellow color.)

The antibody solution is tested:

- With a culture of pertussis bacilli;
- With different antigens, in order to verify the absence of a cross reaction;
- By finding its titer, since it is of interest to use the dilution necessary for obtaining an optimal fluorescence.

4) The smear is then washed in three baths of buffered physiological saline (pH 7.0), each washing lasting two minutes, with constant agitation of the liquid.

5) The slide is allowed to dry for ten minutes in an oven at 37°.

6) The smear is mounted between slide and slide cover in a drop of glycerol solution (9 parts of glycerol; 1 part of cold buffered physiological solution pH 7.0). It is sealed with colorless varnish.

The slide is examined by means of fluorescence microscopy (the immersion oil need not be fluorescent), with an achromatic immersion lens with an iris of 100 x magnification, and a bright-field condenser, a pair of eyepieces of magnification x 10 (the microscope used in this study was a Zeiss microscope with an excitation filter UG 2, which excites the fluorescence of the preparation and is situated between the light source and the condenser of the microscope; and a Wratten 2B stopping filter which serves as a barrier filter placed between the eyepiece and the slide to be examined: It permits the passage of the fluorescent light and eliminates the UV rays).

In the case of a positive reaction, the labeled antibody molecules are absorbed by the homologous antigen and

take the form of antigen particles which appear fluorescent: A characteristic greenish-yellow fluorescence obtains when the illumination is solely by means of intense UV rays (the lamp used was an Hbo 200 (Ostran) very-high pressure mercury-vapor lamp).

When the smears contain Bordetella Pertussis, the bacilli appear in the form of elongated rings which are extremely bright and have a characteristic aspect. The brilliant greenish-yellow halo surrounds a dark center, because there is no fluorescence inside the bacillus.

(The fluorescence of staphylococci is a possibility. It is eliminated by absorbing any of its antibodies by means of a suspension of staphylococci.)

This reaction is specific for Bordetella Pertussis. However, in reality, certain respiratory infections due to Hemophyllus Influenzae may give, on the smear, weakly fluorescent images which may lead to diagnostic errors: These images present themselves in the form of whitish lozenges, sometimes having the appearance of a thick ring with a dark center of unequal size; they are not highly fluorescent and are scattered over the slide.

By contrast, Bordetella Parapertussis does not give a cross-staining with Bordetella Pertussis, and hence cannot appear on the slide.

This reaction is positive in subjects suffering from pertussis who have been vaccinated or who have received gamma globulins specific to pertussis a few days before, but the Bordetella Pertussis appears in one of three forms:

-- In small numbers, exhibiting the usual characteristics but having a weak fluorescence;

-- In the form of small spherical dots of thickened periphery, which are not too bright and whose center is rarely visible;

-- In the form of large elongated rings of twice the usually observed size: The rings are fluorescent and surround a dark center.

In the department of Prof. Julien Marie we have applied this method to a thousand suspected cases of pertussis. The results were as follows:

-- 475 cases have presented an authentic pertussis. The method of staining with fluorescent antibodies indicated a positive diagnosis in 414 cases, or 87%. However, these results differ according to the stage of the disease in which the samples are taken. In effect we have obtained 95% positive results during the catarrhal period of pertussis (out of 153 cases); 91% positive results during the first week of the coughing fits (out of 157 cases); 75% positive results after the first week of the coughing fits (out of 165 cases).

-- Later the positive results decrease rapidly, because the fluorescence of *Bordetella Pertussis* decreases.

In conclusion, the fluorescent antibody technique makes it possible to establish the diagnosis of pertussis during the catarrhal period of the disease -- a period in which the disease is particularly difficult to identify -- in less than one hour. This technique is more sensitive than the method of cultivating nasopharyngeal mucus on Bordet-Gengou's medium during the catarrhal period (95% compared with 86%). However, after this period this technique must always be accompanied by a bacteriological diagnosis which becomes more sensitive after the first week of coughing fits (85% compared with 75%).

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